

**A DISSERTATION ON**  
**ROLE OF IMPRINT CYTOLOGY IN INTRAOPERATIVE**  
**PATHOLOGICAL DIAGNOSIS**

**SUBMITTED TO**

**THE TAMILNADU DR.M.G.R.MEDICAL UNIVERSITY**  
**IN PARTIAL FULFILMENT OF THE REQUIREMENT**  
**FOR THE AWARD OF DEGREE OF**

**M.Ch., (SURGICAL ONCOLOGY)**



**KILPAUK MEDICAL COLLEGE**  
**THE TAMILNADU Dr.M.G.R.MEDICAL UNIVERSITY**  
**CHENNAI, TAMILNADU**

**FEBRUARY 2006**

## **BONAFIDE CERTIFICATE**

This is to Certify that **Dr.S.G.BALAMURUGAN**, bonafide student of M.Ch., Surgical Oncology. (June 2003 to February 2006) in the Department of Surgical Oncology, Government Royapettah Hospital, Chennai - 600 014. has done this dissertation on **“ROLE OF IMPRINT CYTOLOGY IN INTRAOPERATIVE PATHOLOGICAL DIAGNOSIS”** under my guidance and supervision in partial fulfillment of the regulations laid down by The Tamilnadu Dr. M.G.R. Medical University, Chennai for M.Ch. Surgical Oncology Examination to be held in February 2006.

**Prof.G.Illangovan,**  
**M.D., DD., DIH., Ph.D.,**  
DEAN,  
KILPAUK MEDICAL COLLEGE,  
CHENNAI.

**Prof.R.Rajaraman,**  
**M.S., M.Ch.,**  
PROF. & HEAD  
DEPT.OF SURGICAL ONCOLOGY,  
GOVT. ROYAPETTAH HOSPITAL,  
CHENNAI.

## ACKNOWLEDGEMENT

I wish to acknowledge my indebtedness to all those who have been helpful in compiling this dissertation.

It is my pleasure and privilege to record my deep sense of gratitude to **Prof. Dr. R. Rajaraman. M.S., M.Ch.**, Professor & Head, Department of Surgical Oncology, Government Royapettah Hospital, Kilpauk Medical College, Chennai, for his constant encouragement, motivation and guidance given to me in bringing forth this piece of work.

I am extremely grateful to **Dr. S Jegadesh Chandra Bose M.S., M.Ch.**, Asst. Professor, of our Department for his constant support, valuable comments and suggestions in every phase of the study.

My utmost thanks and gratitude to **Dr.S.Mary Lilly, MD.**, Professor, Department of Oncopathology, Government Royapettah Hospital, whose sustained support, skill and expertise in the field of Touch Imprint has been invaluable to me.

I owe my gratitude to Pathology post graduates and clinical staff for their unstinting assistance to complete the study.

Special gratitude is due to my Assistant Professors of our department, **Dr. M.P. Viswanathan M.S., M.Ch.**, and **Dr.S. Balasubramanian M.S., MCh.**, for their help and kindness rendered

I shall be failing in my duty if I do not thank my fellow Post graduates and Technical staff and Para Medical staff for their generous assistance throughout this study.

## **CONTENTS**

- 1. INTRODUCTION**
- 2. AIM**
- 3. REVIEW OF LITERATURE**
- 4. MATERIALS AND METHODS**
- 5. RESULTS AND OBSERVATIONS**
- 6. DISCUSSION**
- 7. CONCLUSION**
- 8. PROFORMA**
- 9. BIBLIOGRAPHY**

# **INTRODUCTION**

## INTRODUCTION

The development of the concept of intraoperative evaluation of tissue diagnosis had revolutionary effect in the field of surgical oncology.

Intraoperative tissue diagnosis helps cancer surgeon

1. To confirm pathological diagnosis when pre-operative diagnosis is inconclusive.
2. To decide about the extent of the surgery
  - a. To avoid nodal dissection when sentinel node of nodal basin is negative and to complete nodal dissection when sentinel node is positive
  - b. To assess the marginal status after excision and help to achieve margin free excisions
3. To avoid the major ablative surgeries when, tumor has metastasized beyond a proposed resection.
4. To decide the definitive procedure after needle localization biopsy in suspected Ca. Breast.

Currently intra operative evaluation is performed using

1. Frozen section
2. Imprint cytology
3. Immuno Histo Chemistry
4. Molecular study - R.T PCR

Touch imprint cytology is simple, quick and inexpensive and can be done even in centers where only basic pathology facility is available. Secondly the low cost and rapidity of this procedure is a big boon to the community based hospitals.

Imprint cytodiagnosis is a relatively new technique that was introduced by Dudgeon & Patrick from UK and subsequently tried by several others in the last decade. The technique is relatively simple, cheap, reliable and does not require elaborate apparatus. The time required for imprinting a slide, staining and obtaining a readable result is only 6-8 minutes. Thus it is suggested, that in places where frozen section facilities are not available, imprint cytodiagnosis would be a valuable alternative.

The mean accuracy rate for imprint cytology is 91% with range from 78% to 98% in various literatures.

The present study is mainly designed to determine the reliability of this procedure and to compare our results with literature.

**AIM**



## AIM

Touch imprint cytology is a simple and inexpensive method of detecting nodal metastatic disease even in centers, which do not have frozen section in their diagnostic armamentarium. Imprint cytology is reported to have a greater accuracy than frozen section since the latter is associated with higher false negative rate (25%).

The aim of the present study is to evaluate our institutional experience with touch imprint cytology and to ascertain the reliability of touch imprint cytology as an intraoperative diagnostic tool in determining the histologic status of suspicious lymph nodes for metastasis and there by determining its sensitivity, accuracy, predictive value and its feasibility in our institution to aid the surgical oncologic procedures.

However the present study is designed to assess the value of touch imprint cytodiagnosis in solid tumors by extrapolating its rich sentinel node experience from the many well-known studies.

## **REVIEW OF LITERATURE**

## **REVIEW OF LITERATURE**

Modern technique of intraoperative tissue diagnosis is one of the useful additions to the armamentarium of the cancer surgeon.

It helps to assess the extent of disease, whether or not tumor has metastasized beyond a proposed resection.

### **NODAL MESTASTASIS**

In solid tumors, clinical nodal staging is a major challenge. Clinical examination is influenced by the skill of the examiner, the patient's body habitus and status of previous treatment like surgery, radiation and chemotherapy. As a result of these factors, the false negative rate in clinical assessment ranges from 20-30%. Imaging techniques such as CT scanning, MRI, US has been used for evaluation. The sensitivity of CT scanning is 81%. Size of the nodes and central lucency of nodes have been used as criteria for identifying positive nodes but there is a low specificity. Routine scanning of lymphatic basin is not justifiable at present. U.S. and with U.S. guided Fine Needle Aspiration, the accuracy has improved to 89% but it is highly operator dependent. No pre-treatment study can accurately assess or replace the histopathology. Hence the goal of identifying the sub clinical disease without surgical intervention remains elusive.

It is well known that 20% to 40% of patients with solid tumor who have no palpable disease in their lymphatic basin will harbor occult disease. It is therefore, therapeutically tempting to treat these patients on the basis that this will avoid subsequent treatment, which may not be as effective. It means that 60%-80% of patients are "over-treated" and exposed to unnecessary morbidity and even mortality.

### **OPTIONS OF THE MANAGEMENT OF THE N<sub>0</sub> NODE**

1. Wait and watch
2. Prophylactic nodal dissection
3. Intraoperative lymphatic mapping and sentinel node biopsy.
4. Selective lymphadenectomy and intra operative tissue diagnosis

Wait and watch policy is not justifiable

It is well known that only 20% to 40% of patients with solid tumor, who do not have clinically apparent node, will harbor occult disease. Prophylactic Nodal dissection in these patients will result in 60% to 80% of patient getting over-treatment with significant morbidity.

Intraoperative lymphatic mapping (ILM) and selective lymph node dissection (SLND) are revolutionary concepts that, in a short period, have shown the potential to alter dramatically the management of many patients with solid neoplasms. The rapid adoption of this approach to staging of solid neoplasms by the surgical oncology community has resulted in an explosion of data on this subject.

Despite lack of standardization of this technique, debate regarding the operative definition of a sentinel node and even the appropriateness of this approach outside of research settings, this approach continues. All reports from a variety of clinical settings, to date, however, support the original sentinel node hypothesis. Important issues remain unresolved, including standardization issues and the biologic relevance of immunohistochemical findings. It is apparent, however, that ILM provides greater diagnostic accuracy and lower morbidity, as substantial numbers of truly node-negative patients with cutaneous melanoma and breast cancer can be spared the morbidity of a regional node dissection that has no potential therapeutic value.

In community hospitals like our institution where facilities for intraoperative lymphatic mapping and sentinel node biopsy are not available, the alternative approach is selective lymphadenectomy and submission of the specimen for intraoperative pathological examination followed by radical nodal dissection if nodes were positive.

## **CLINICAL APPLICATIONS**

### **HEAD AND NECK CANCER**

Comprehensive neck dissection is the standard treatment for obvious nodal disease in the neck. With emphasis on function preservation and cosmesis in addition to achieving adequate local disease control, conservative neck dissections have gained popularity. Growing historical evidence suggests

that modified and selective neck dissections offer disease control comparable to radical neck dissection but with less morbidity.

A standard RND has no role in the management of patients with N0 neck status. A SND, or rarely a MRND, would be required. A recent study has shown comparable recurrence rates for RND compared with SND with there being no statistically significant difference. It has to be emphasized that an END procedure must be "individualized". The decision regarding the type of neck dissection depends on the site of primary tumor, other tumor factors (e.g. location, depth, size, differentiation, vascular or perineural invasion), the patient and the surgeon undertaking the procedure. Tumors of the tongue, floor of mouth, nasopharynx, oropharynx, hypopharynx and supraglottic larynx have a high incidence of nodal metastases. In contrast, tumors of the buccal mucosa, lip, paranasal sinuses and glottic larynx metastasize less frequently. For a patient who stays some distance from the surgical centre and who is not expected to come for regular follow-up (and for unreliable patients) an MRND would be a better option.

Asthana et al evaluated a novel method of intraoperative staging using sentinel node biopsy and intraoperative imprint cytology in 32 patients with head and neck cancer. Intraoperative imprint cytology (IIC) could accurately predict the false - negative result. The overall sensitivity, specificity, and accuracy of IIC were 87.5% 95.4% and 93.3% respectively.

## **BREAST CANCER**

In the absence of metastatic disease, the single most predictor of outcome for women with breast carcinoma is the status of the regional lymph nodes. Traditionally, in patients with carcinoma of breast, axillary lymph node status has been evaluated by routine axillary lymph node dissection (ALND) with lumpectomy or mastectomy. ALND is used to obtain precise staging data, provide local control and for selection of adjuvant therapy. Unfortunately, the only patients who are likely to derive therapeutic benefit from ALND are patients with positive nodes. ALND is associated with considerable morbidity including lymphedema, neurological abnormalities principally to the intercosto-brachial nerves, shoulder stiffness and rarely angiosarcoma. The sentinel lymph node biopsy is an accurate predictor of the overall axillary nodal status and has both high sensitivity and specificity, especially T<sub>1</sub>, T<sub>2</sub> tumors with no palpable axillary node.

The key advantage of intraoperative analysis of lymph nodes is the ability to stage patients during the initial operation while the patient is undergoing the surgical resection of the primary tumor. Intraoperative analysis of lymph nodes is clearly desirable as the surgeon may proceed with axillary dissection during a single procedure if the nodes are positive. The alternative would be to terminate the procedure after the sentinel lymph node biopsy is performed with the intention of returning to the operating room for a second procedure if the permanent histological evaluation confirms the presence of metastatic carcinoma. The second operative procedure likely results in higher costs and clearly results in increased patient anxiety and discomfort. High

levels of diagnostic accuracy are required for this one-stage procedure, as a false positive intraoperative diagnosis would lead to potentially unnecessary block dissection with its attendant morbidity.

Traditionally, frozen section evaluation has been the technique of choice for intraoperative tissue evaluation. Numerous studies of frozen section have been reported evaluating the utility of this technique for the intraoperative analysis of sentinel lymph nodes with rare false positives reported. Sensitivity has been quite variable ranging from less than 60% and up to 95% and number of factors contributes to this variability, including the size of the primary lesion, size and focality of metastasis, type of primary carcinoma and the number of frozen sections evaluated.

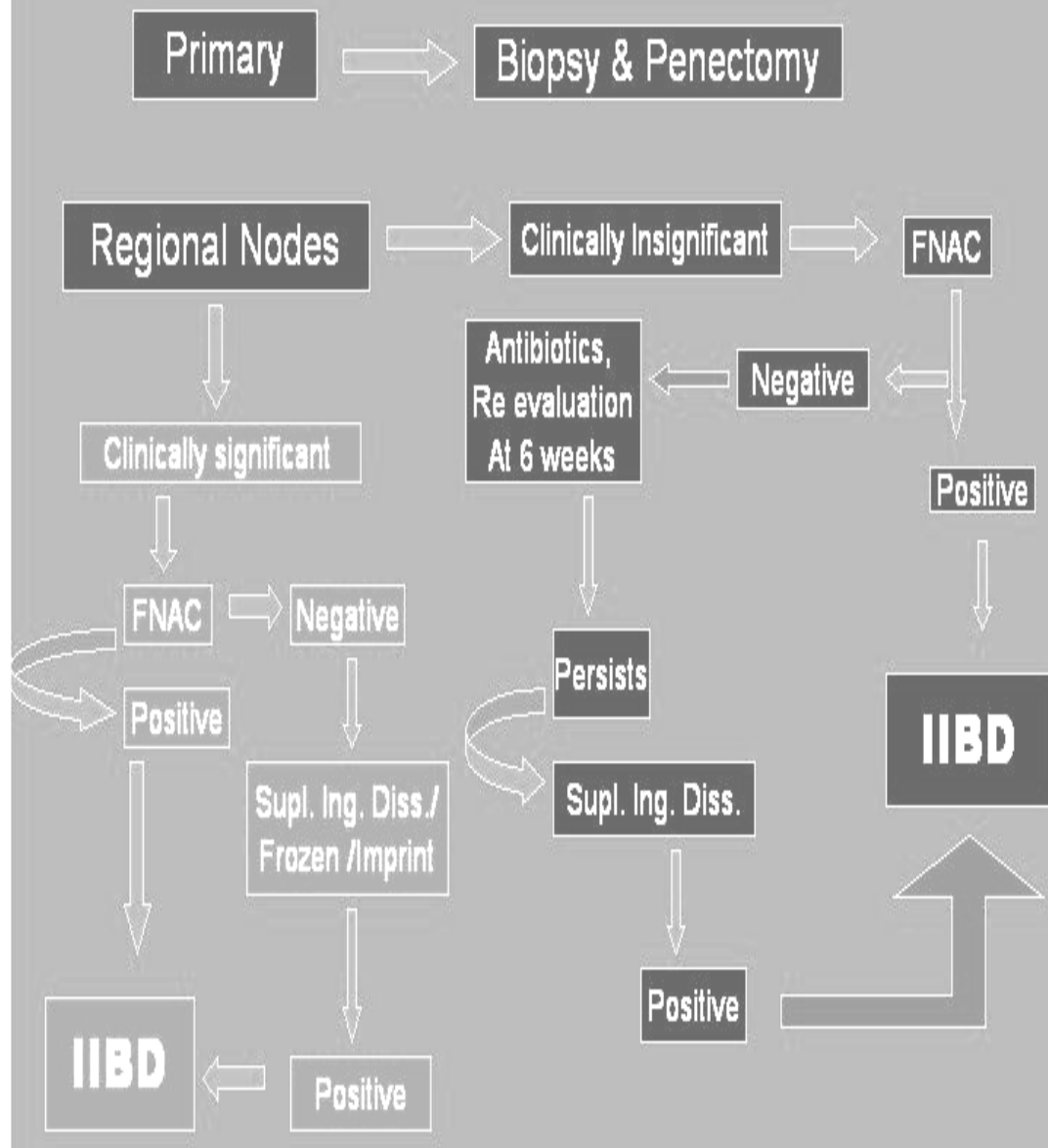
## **CARCINOMA PENIS**

The presence and extent of inguinal lymph node metastasis are the most important prognostic factors. In the absence of inguinal nodal metastasis, pelvic nodal metastasis is rare. Inguinal metastasis is potentially curable by lymphadenectomy alone. Pelvic nodal metastasis is often incurable. 20% of clinically negative nodes harbor occult lymphatic metastasis.

Standard management of nodal disease is Ilio-Inguinal Block Dissection. It is associated with considerable chronic morbidity including skin necrosis, lymphedema, wound infection, seroma and femoral hernia.



## Regional Nodes in Ca. Penis - Management Protocol



## **GASTROINTESTINAL MALIGNANCY**

Extent of Lymphadenectomy in gastric Cancer is debatable.

D<sub>2</sub> Lymphadenectomy has no survival benefit in western series. In contrast, Japanese results have definitive survival benefit. N<sub>3</sub> Nodes - Para Aortic, mesenteric and Retro Pancreatic Nodes are considered as metastatic disease. Involvement of these Nodes is incurable.

In Colon Cancer, involvement of principal Nodes- S.M.A, I.M.A. Nodes are incurable.

Prem Sharma et al., correlating with definite histology work up, studied imprint Cytology in the diagnosis of gastrointestinal tract malignancies. They found that overall diagnostic accuracy of histology and Imprint Cytology in esophagus, stomach and lower gastrointestinal tract was 95%, 98%, 98% and 95%, 100%, 98% respectively. When combined, the diagnostic accuracy increased to 98%, 100% and 100% in esophagus, stomach and lower gastrointestinal tract respectively.

## **GYNECOLOGICAL CANCER**

In Ca. Cervix, pelvic nodal metastasis is associated with poor prognosis. It warrants adjuvant radiotherapy. Involvement of para aortic node is incurable. Radical surgeries like Wertheim's hysterectomy and pelvic exenteration are contra indicated when Para aortic nodes are involved.

Bhabra et al, from U.K described the value of the technique applied to intraoperative diagnosis of lymph node metastases in Gynecological malignancy. 475 Lymph nodes were examined using Intraoperative tissue diagnosis followed by routine histology. The technique of Intraoperative tissue diagnosis was found to have zero false negative rates, 0.6% false positive rate and accuracy of 99.3%.

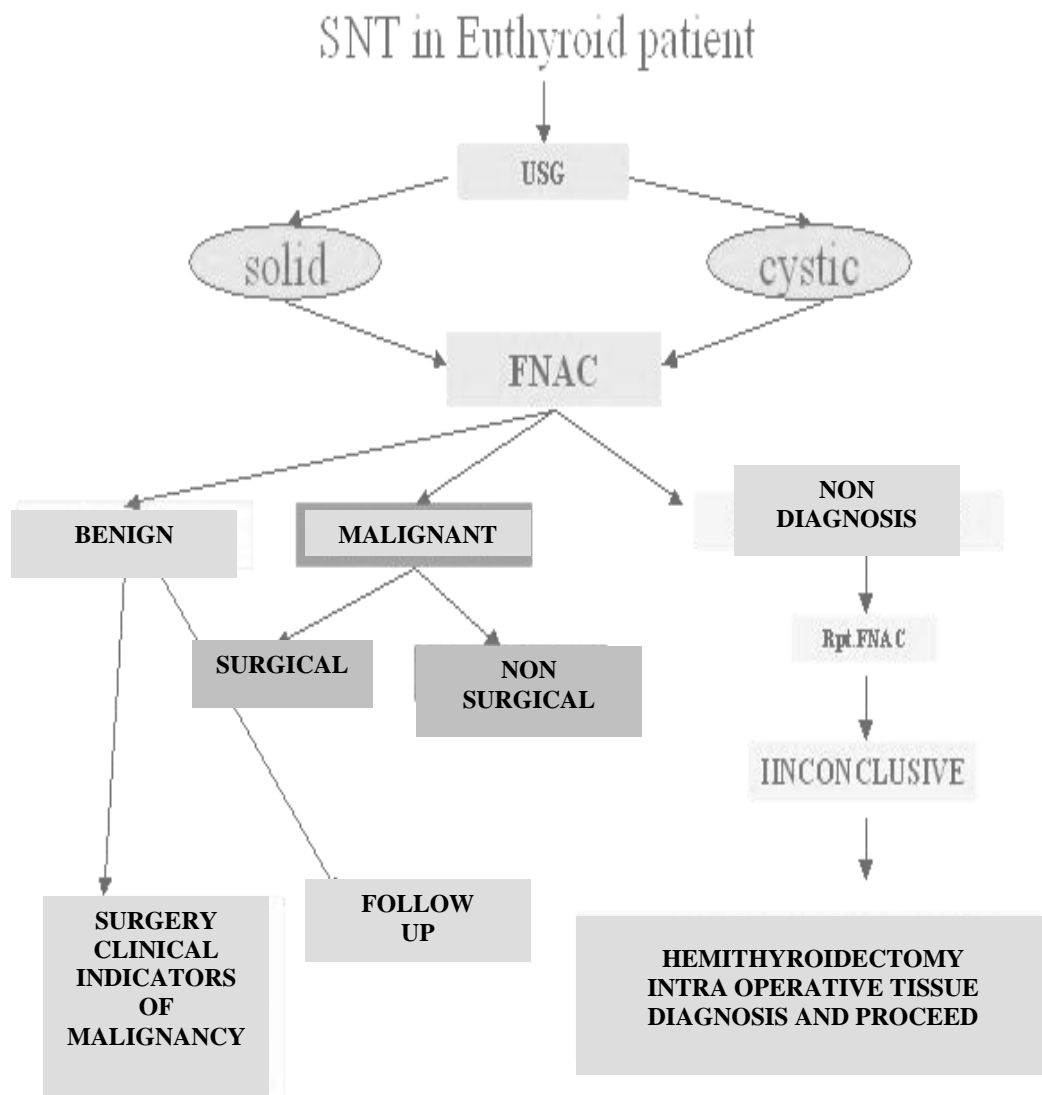
### **THYROID CANCER**

In solitary thyroid nodule, to differentiate between benign and malignant swellings, FNAC is helpful. The sensitivity is 90%. But accuracy of imprint cytology is greater than FNAC. Imprint cytology is not useful to differentiate between follicular adenoma and Carcinoma, where histology is a must to examine capsular and vascular invasion

### **NEEDLE LOCALISED BREAST BIOPSIES**

Shabaik et al., studied the relevance of Imprint Cytology in 503 needle localized breast biopsies and they concluded that intraoperative evaluation of needle localized breast biopsy can provide the surgeon with information useful to immediate clinical management and also indicated that while Imprint cytology and Frozen Section are occasionally complementary, increased reliability in Intraoperative cytological diagnosis will allow the surgical pathologists to conduct diagnostic and prognostic studies on artifact free and intact lesional tissues.

## S.N.T MANAGEMENT



## **IMPRINT CYTOLOGY IN SURGICAL MARGINS**

Andrew J. Creager et al. extended the Imprint Cytology technique for evaluating surgical margins intraoperatively to achieve margin free excisions to be performed during the initial surgery. He concluded that Imprint Cytology evaluation in breast conservation therapy is a simple, rapid, accurate and cost effective method for intraoperative determination of margin status that can be reproduced in the community hospital setting. It allows for evaluation of the entire surface area of the lumpectomy specimen, whereas such evaluation is impractical using Frozen Section because of time restrictions and the technical challenges associated within cutting frozen sections of adipose tissue, especially if extensive calcifications are present. Finally, Imprint Cytology may reduce local recurrence rates and improve cosmesis in patients undergoing breast conservation treatment.

## **OPTIONS—INTRA OPERATIVE TISSUE EVALUATION**

1. Frozen section
2. Imprint cytology
3. ImmunoHistoChemistry.
4. Molecular study – RT PCR

## **FROZEN SECTION**

### **ADVANTAGES OF FROZEN SECTION ANALYSIS**

Maintenance of normal lymph node architecture and familiarity of pathologists with the technique with preserved architecture, pathologists may pay added attention to regions such as the subcapsular sinuses an area with a known predilection for harbouring metastasis.

### **DISADVANTAGES OF FROZEN SECTION ANALYSIS**

Unfortunately Frozen section analysis has several disadvantages. Sectioning the node in the cryostat may consume large amounts of tissue that are unfortunately lost for future evaluation. In addition, freezing artifact may be produced both in the Frozen section itself and in subsequent permanent sections from the same block. Thus, the probability of missing micrometastases is increased due to tissue loss and there is interpretation difficulty secondary to freezing artifact.

The size of the lymph node is a factor that can affect intraoperative evaluation of the nodal metastasis. This problem usually affects frozen section evaluation because it can be difficult to cut the large pieces of lymph node onto a glass slide especially when partially or wholly replaced by fat. There are no such limitations with Intraoperative Imprint Cytology

Although, intra operative examination of the sentinel lymph node may avoid the cost and operative morbidity of a separate surgical operation when the node shows tumor cells, previous sentinel lymph node studies with frozen section have reported variable results.

Frozen section histological examination of lymph nodes is known to have a high false negative rate of upto 27%. Imprint cytology has been reported to have a lower false rate with the sensitivity reaching 100% in some studies.

## **IMPRINT CYTOLOGY**

Imprint cytology is for many reasons, a much more practical method with which to evaluate lymph nodes intraoperatively and it appears to be a viable alternative to frozen sectioning. Cost and rapidity of intra operative analysis are other factors that must be considered. At our institution, intraoperative touch preparation analysis can be performed quickly and at a substantially lower cost than traditional frozen section histology. The cost of frozen section is significantly greater than intraoperative Imprint Cytology.

One study, however, noted a false positive result (1%) on intraoperative Imprint cytology a potential problem that also has been observed using Imprint cytology of low axillary lymph nodes sample. Although a rare false positive result could occur with frozen section examination, it has not yet been described and seems to be a potentially greater problem with Imprint cytology techniques.

To minimize the possibility of false - positive results, intraoperative indeterminate results (atypical/suspicious) results, which occurred during study are regarded as negative at the time of surgery and at final diagnosis, unless confirmed positive on H & E. In indeterminate results, the reactive histocytes, lymphocytes, and endothelial cells may appear atypical and occasionally difficult to distinguish from a micrometastasis. This is a greater problem with Imprint cytology.

The possible reasons for the false positive result are.

1. First the Imprint cytology specimen contained the only metastatic deposit in the part of the lymph node that was lost in the deeper sections of the lymph node and consequently not found on final histopathology.
2. Second the specimen could be contaminated as reported also in few studies.

## **ACCURACY OF INTRAOPERATIVE RESULTS**

The accuracy for Imprint cytology ranges from 78 to 98% with a mean of 91%. The mean accuracy for frozen section in literature is 89% with a range from 83 to 96%.

## **INTRAOPERATIVE TIME FOR TOUCH IMPRINT CYTOLOGY**

A median waiting time of 25 min. for the results in the present study is acceptable and allows the surgeon to perform definitive surgery on the primary tumor while awaiting results of the Imprint cytology.



## **FACTORS MODIFYING THE OUTCOME OF THE RESULTS**

Direct comparisons between series published by different institutions are problematic due to difference in processing methodologies, patient populations, and outcome measures. Many factors can affect intraoperative touch preparation accuracies including reagents, the number of lymph nodes evaluated, the number of node sections made and the time constraints placed upon the pathology team. Among these are the proportions of patients who have lymph node positive disease versus early stage disease, the prevalence of solely micrometastatic disease, and the prevalence of invasive lobular patients, whose histology is generally accepted to be more difficult to evaluate intraoperatively.

## **REPORTING SYSTEM**

Reporting of the imprint cytology should be quick and accurate. False positivity leads to unnecessary mutilating and irreversible surgical procedure and false negativity leads to unnecessary delay for second operation.

Report should be as little as possible and as much as needed. Heroic demonstration of pathologist skill is not required.

The lab should be in the same building where operation theatre is located.

Imprint cytology processing is an emergency to pathologist who is normally not familiar with daily stress of surgeon.

### **Touch Imprint cytology Versus Frozen section**

	<b>Touch Imprint Cytology</b>	<b>Frozen Section</b>
1.	Rapid (< 10min)	Time consuming (30 min)
2.	Inexpensive	More expensive
3.	Preserve tissues	50% tissue lost
4.	Highly specific	Less specific
5.	No artifacts	Freeze thaw artifacts
6.	Easy to perform multiple slides	Serial sectioning very time consuming

The advantages of Touch Imprint cytology over frozen section are numerous.

It is quicker; taking about 5-10 min, as compared with more than 20 to 30 min. for frozen section analysis.

Therefore touch preparation can decrease both anaesthetic time and operative time considerably. In addition, Touch Imprint cytology does not have the disadvantage of tissue destruction and freeze thaw artifact that occurs routinely with the frozen section technique.

### **LYMPH NODE MICROMETASTASIS**

Lymph node metastases were defined as those meaning greater than 2mm in size as macrometastases and those falling short of 2mm size were labelled as micrometastases and compared against the currently accepted standard of H & E histology.

Unfortunately, the related subject of lymph node micro metastasis sometimes clouds the clarity with which the literature speaks on the subject of the significance of occult lymph node metastases in breast cancer, which is somewhat more obscure. Regrettably, many studies use the terms Occult metastases and micrometastases interchangeably, despite the fact that occult metastases often are quite large, replacing considerable Portions of the node with malignant deposits.

Micrometastases, as the name implies, are microscopic deposits of tumor in lymph nodes. There is, however, no consensus on the definition of a micrometastasis, with various publications using upper limits of 0.2 to 2.0 mm and others using definitions based on the area of tumor involvement, such as <20% of the lymph node cross sectional area.

Macrometastases were identified successfully by Imprint cytology and frozen section in 98% of patients.  $\leq 2.0\text{mm}$  detected by Imprint cytology and frozen section in only 28% of cases. When lymph node metastasis is seen, several factors influence the sensitivity of both imprint and frozen section evaluation of sentinel lymph node. The first critical factor is the size of metastasis. In breast carcinoma, the sensitivity of detecting micrometastasis is low because of the focal nature and small size of the metastases. Infact, in an exhaustive Frozen section study of sentinel lymph node in patients with breast carcinoma, Viale et al., sectioned lymph nodes completely intraoperatively, using both H & E staining and intraoperative cytokeratin immuno staining at each level. In that study, the detection of macrometastasis usually occurred after examination of the first Frozen section level. Detection of

micrometastases was more problematic because they tended to be scattered unpredictably throughout the lymph node

## **MICROMETASTASES AND ITS CLINICAL IMPLICATION**

The disadvantage of potentially missing some micrometastasis due to loss of tissue is clearly outweighed by the advantage of performing an axillary lymphadenectomy in the first operation in a sentinel node positive patient.

Shiver et al., also found that Imprint cytology was significantly more sensitive for macrometastasis (87%) than for micrometastasis (22%).

In the study by Lee et al., 83% of false negative Imprint cytology cases were due to micro metastasis.

Llatjos et al., using imprint cytology and rapid cytokeratin immunostaining reported that 80% of their false-negative results were due to micrometastasis. Only one micrometastasis was detected by intraoperative Imprint cytology.

Recently Creager et al. reported that sensitivity for macrometastases was significantly better than for micrometastases (81 and 21% respectively) in a series of 646 patients. Identification of micrometastases is problem not only in Imprint cytology but also in frozen section. This may not be a serious limitation of intraoperative Imprint Cytology, because patients with sentinel node macrometastases can reliably be detected by imprint cytology and are most likely to benefit from complete lymphadenectomy.

The benefit of the completion axillary lymphadenectomy in patients with micrometastatic disease in the sentinel node is unknown and questionable because of the controversial role of regional control on survival and the beneficial effect of adjuvant systematic therapy. In the future, the results of international prospective clinical trials will probably show that a completion axillary lymph node dissection will not be necessary for micrometastatic sentinel node disease.

Although touch preparations have a false negative rate of 4.9% this is likely not to be clinically significant, as it represents micrometastatic disease, which carries a low likelihood of additional nodal pathology. Therefore in this subset of patients, a "MISS" on touch imprint evaluation does not usually translate into a return to the operative suite

## **REVIEW OF INTRA OPERATIVE TOUCH IMPRINT CYTOLOGY RESULTS**

The need for the intraoperative assessment of nodal metastasis seems justified, as it may obviate the need for a second operation and this is bolstered by findings from Motomora et al., reported the results of Imprint cytological examination of sentinel lymph nodes with 90.9%. Sensitivity, 98.5% specificity and 96% overall accuracy and for Frozen section were 52% sensitivity, 100% specificity, 88% accuracy. He concluded that Imprint cytology could detect micrometastasis more accurately than the conventional H & E staining sectioning. Scerni et al. demonstrated the imprint analysis had a sensitivity of 83% and a negative predictive value of 86%. False positive cases rarely occur with trained cytopathologists, and only one such a case was reported by Ku et al.

Karamlou et al., had shown that appraisal of the sentinel lymph node analysing touch imprint cytology will accurately predict a positive axillary node with a positive predictive value of 100% and a negative predictive value of 95% and so, would recommend that all patients with a positive touch preparation result have complete axillary dissection, as they had no false positives in our experience. Although the Touch Imprint Cytology technique had a false negative rate of 4.9%, he believed that it still leads to clinically appropriate management of the axilla. The false negative results all occurred in the setting of micrometastases.

Veronesi et al., reported sensitivity and specificity of more than 90% for intraoperative frozen section analysis of sentinel nodes with the use of multiple sections involving most of nodal tissue and with intraoperative pathology time in excess of 40 minutes. Although this technique is possible, it is not feasible for many centers in terms of time and cost, and it can be performed only at the expense of loss of all nodal tissue for subsequent analysis. Other groups performing less complex frozen section analysis have been unable to duplicate these results although Van Diest et al., also achieved high accuracy with Frozen section with less tissue analysed and with shorter processing times, they thought this procedure to be preferable to Imprint cytology.

The largest series by Henry - Tillman et al., involved 479 sentinel lymph nodes and 247 patients. The authors reported a “per node sensitivity” of 94% and a positive predictive value of 98% on a “per patient” basis, sensitivity was 91%<sup>5</sup>. Other authors also report similar sensitivities. However in many of these series, touch preparation technology was evaluated to determine the diagnostic accuracy of the test itself and not to influence on intraoperative decision for possible immediate axillary lymphadenectomy.

## **MATERIALS AND METHODS**

## **MATERIALS AND METHODS**

We examined 70 samples in 45 patients with various tumors in this study between July 03 and Sep. 2005. There was no age restriction and patient age ranged from 27 to 70 years.

A Retrospective Analysis of 44 pts whose surgical procedures were based on imprint cytology, were analyzed.

Oral cancers - 5 Cases - with clinically negative nodes with having high risk for nodal metastasis was treated by supraomohyoid Block dissection and then submitted for imprint cytology. R.N.D. was done for all patients with positive results.

Ca penis- 6 cases-and skin tumors in lower limb -2 cases - with palpable inguinal nodes after antibiotic treatment was treated by Superficial Inguinal block dissection followed by IIBD based on imprint cytology.

8 cases of Post R.T cervical cancer with adjacent organ invasion were selected for pelvic exenteration provided if para aortic nodal - Imprint Cytology is negative.

In 6 cases of Thyroid Solitary Nodules was treated by hemi thyroidectomy and imprint cytology followed by total thyroidectomy if it is positive.



In 3 cases of gastric cancer, Para aortic nodal sampling and imprint cytology was done to decide D<sub>2</sub> gastrectomy only if it is negative.

In 9 cases of Ca breast after Axillary dissection the nodes were submitted for imprint cytology to compare with HPE report.

In 3 cases of Lymphoma after nodal Biopsy, the result of both imprint and HPE were compared.

Testicular cancer with retroperitoneal nodes (1 case) after salvage chemotherapy, it was submitted imprint cytology to compare with HPE report.

After the surgical procedures were performed the specimen was submitted for evaluating the pathological status by touch imprint cytodiagnosis, followed by definite paraffin sections. The minimum size of the node as inclusive criteria into the study was prefixed as 1 cm.

The clinical details and other relevant findings regarding the patient were informed priorly to the attending pathologist to hasten up the intraoperative pathologic processing period.

As soon as suspicious lymph nodes were excised they were carefully labeled and delivered fresh to the room adjacent to the operating suite.

### **Intraoperative Pathologic Evaluation**

The freshly received lymph node initially was measured and fibro fatty tissue surrounding the lymph node was removed accurately without disturbing

the lymph node capsule, before bisecting the lymph node along its long axis. Care was taken to obtain complete cross sections of the maximum dimension, preferably including the hilum and the marginal sinus.

From the freshly cut, flat surface of specimen, imprints were taken by gently touching the flat surface firmly with a standard glass slide twice or thrice. Two slides were prepared from each specimen.

The imprints from each surface were fixed immediately in alcohol for few minutes and then stained immediately with rapid H & E technique.

#### **Rapid hematoxylin and eosin stain - Standing Procedure**

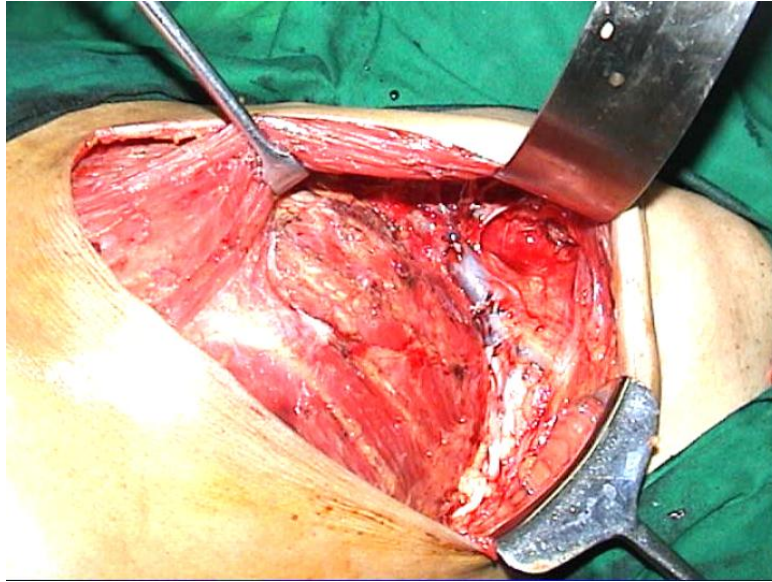
1. Fix smear in 10 per cent neutral buffered formalin at room for 20 seconds.
2. Rinse in tap water
3. Stain in Harris's (Progressive) hematoxylin for 1 minute.
4. Wash well in tap water for 10 - 20 seconds.
5. Stain in 1 per cent aqueous eosin for 10 seconds.
6. Rinse in tap water.
7. Dehydrate, clear and mount

Single experienced pathologist reported all the slides. The diagnosis was rendered in an average of 20 minutes and communicated to the Surgeon Via Telephone or in person.

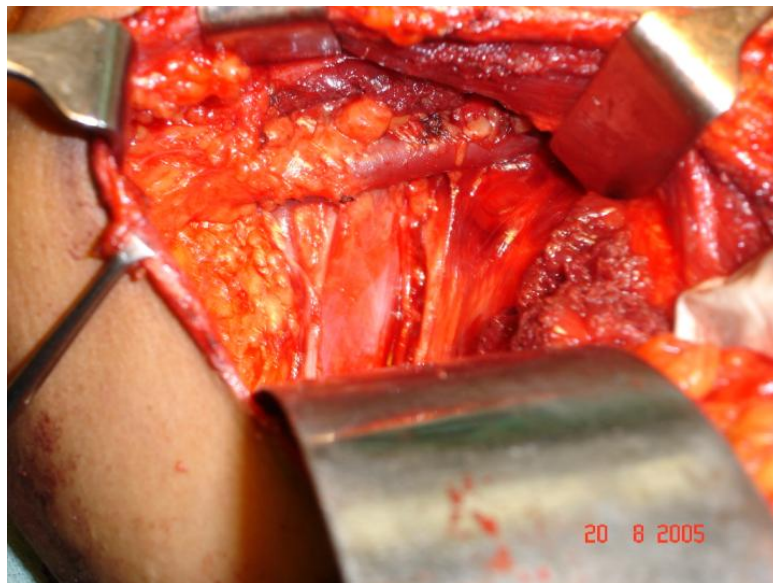
Diagnostic categories use in reporting cytological finding included.

1. Negative for malignancy.
2. Atypical cells seen, suspicious for malignancy.
3. Positive for malignancy.

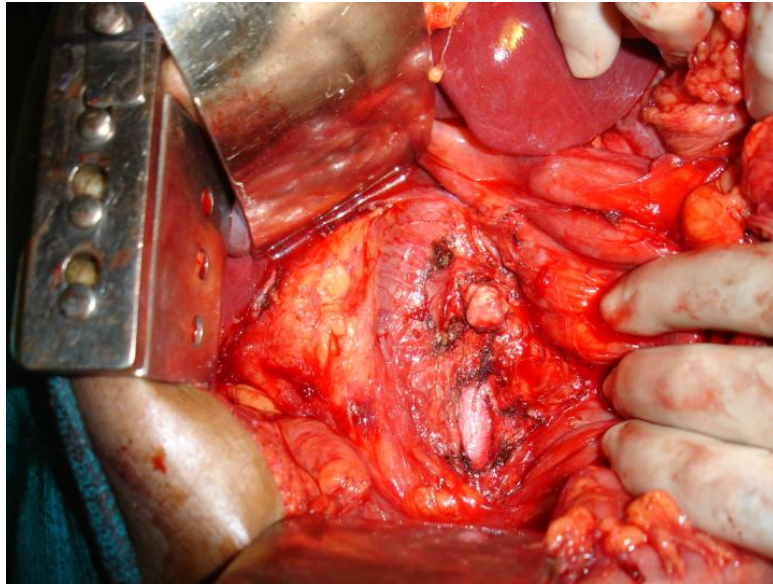
Permanent Section slides were prepared as routine for all specimens and were reported by same pathologist.



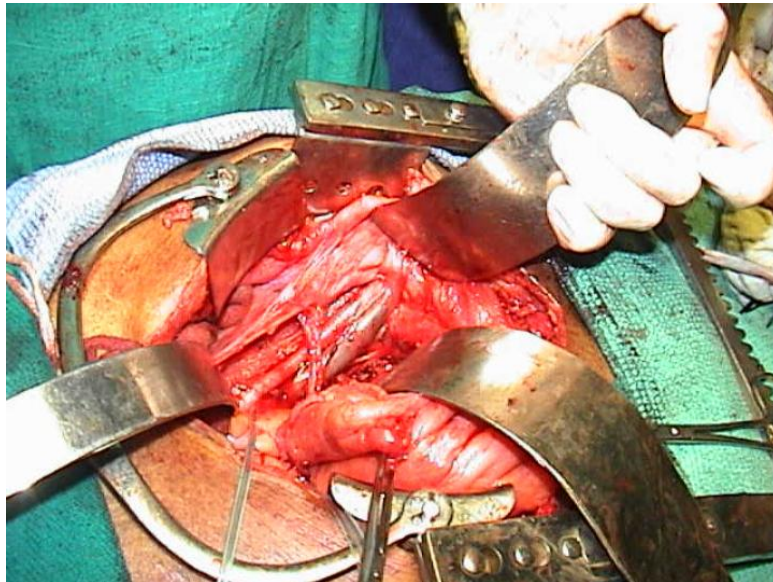
**LT. AXILLARY DISSECTION**



**RT. AXILLARY DISSECTION**

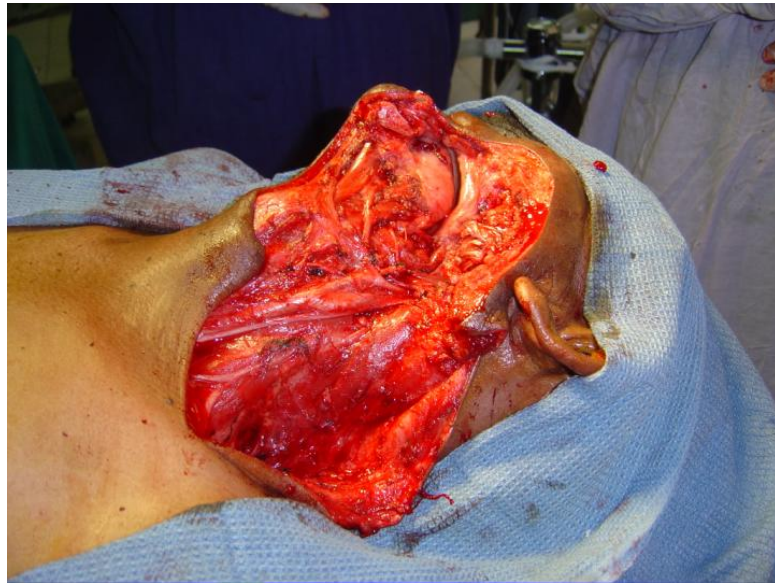


**PARA - AORTIC NODAL DISSECTION**

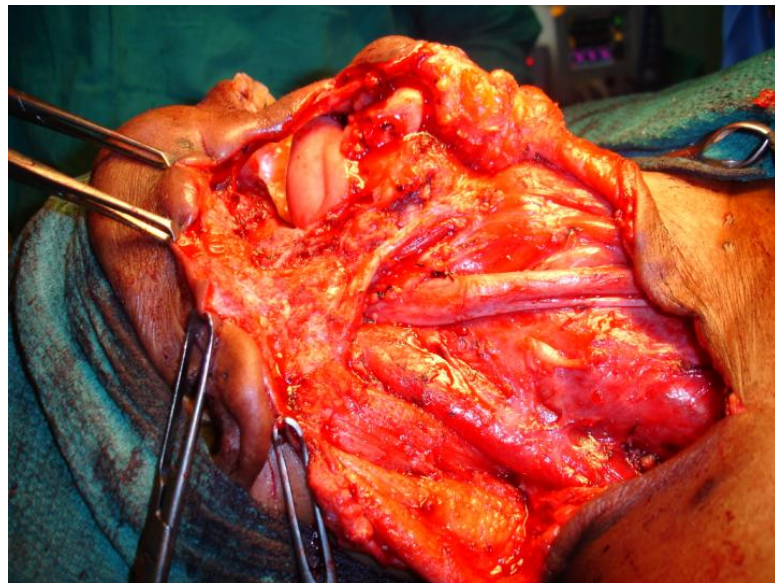


**PELVIC NODAL DISSECTION**

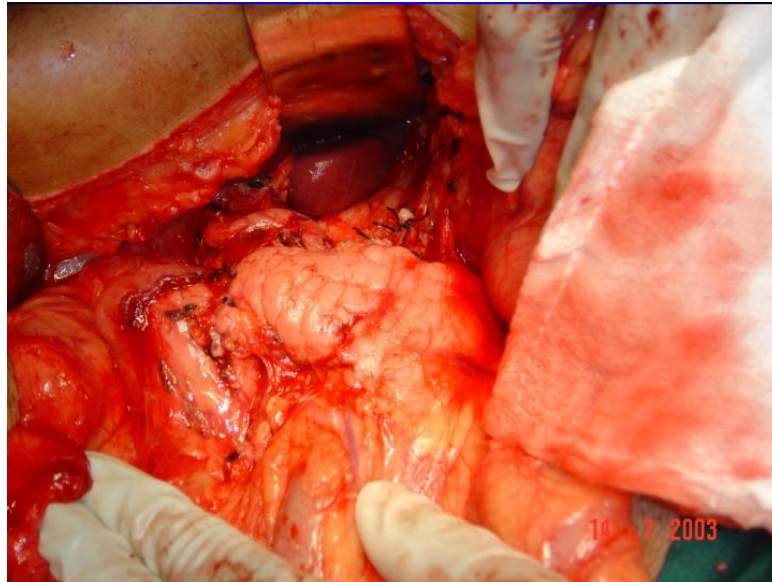




**LT- COMPOSITE DISSECTION**



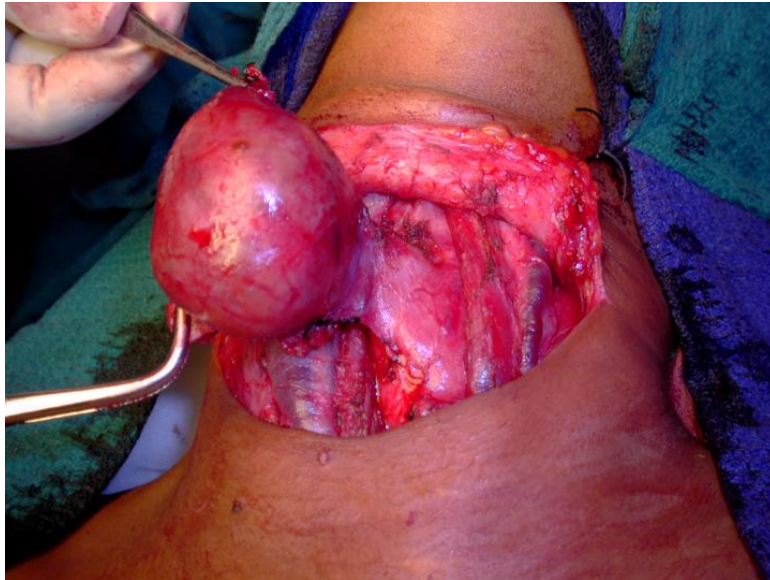
**RT- COMPOSITE DISSECTION**



**D2 GASTRECTOMY**



**D2 GASTRECTOMY SPECIMEN**



**RT- HEMITHYROIDECTOMY**



**HEMITHYROIDECTOMY - SPECIMEN**





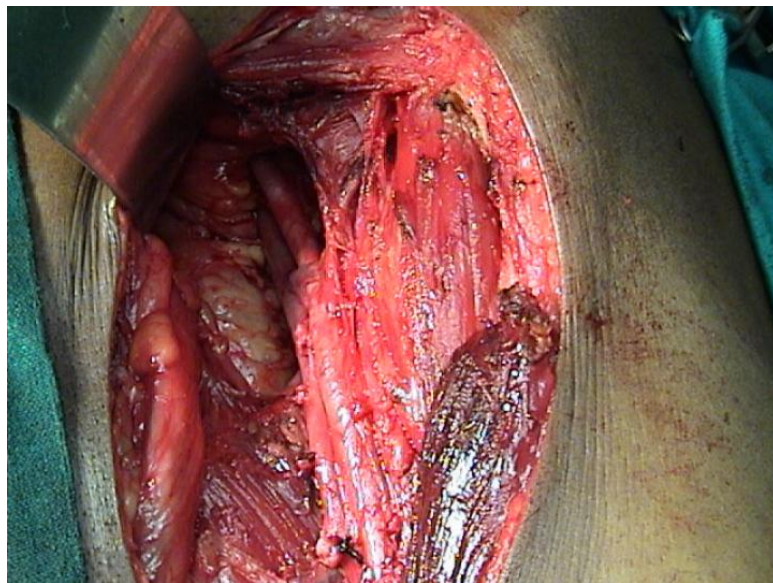
**TOTAL THYROIDECTOMY - SPECIMEN**



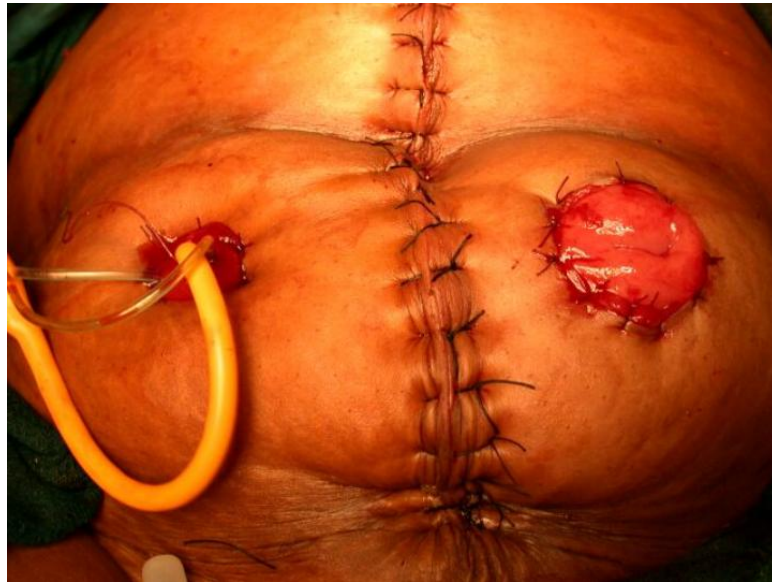
**COMPOSITE RESECTION SPECIMEN**



**SUPERFICIAL INGUINAL  
BLOCK DISSECTION**



**ILIO INGUINAL BLOCK DISSECTION**



**TOTAL PELVIC EXENTERATION –STOMAS**



**TOTAL PELVIC EXENTERATION –SPECIMEN**





**NODE - BEFORE BISECTION**



**BISECTED NODE**



**IMPRINT SMEAR - FROM NODE**



**IMPRINT SMEAR - FROM SPECIMEN**

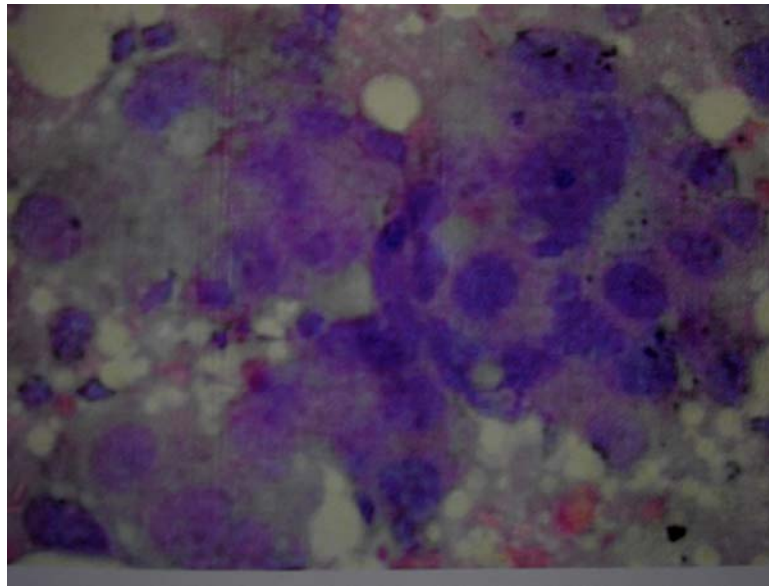


**IMPRINT SMEAR**

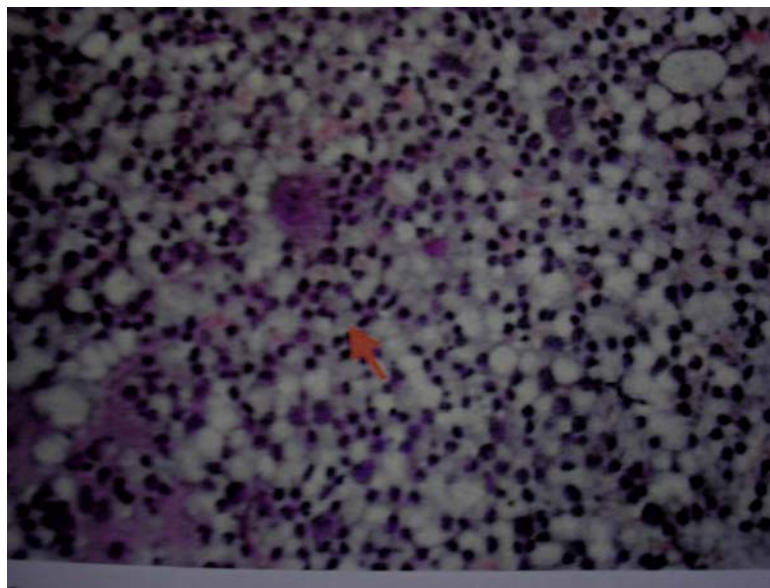


**IMPRINT SMEAR - PROCESSING**

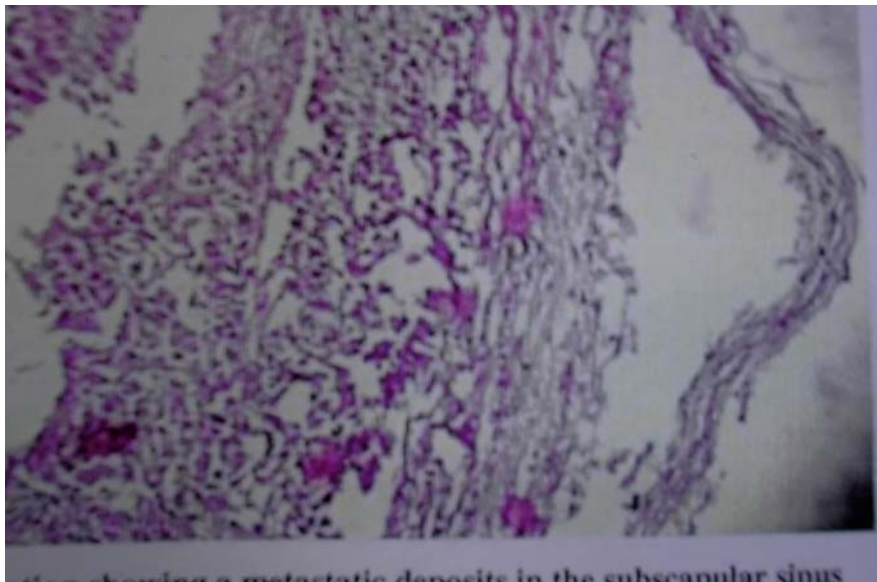




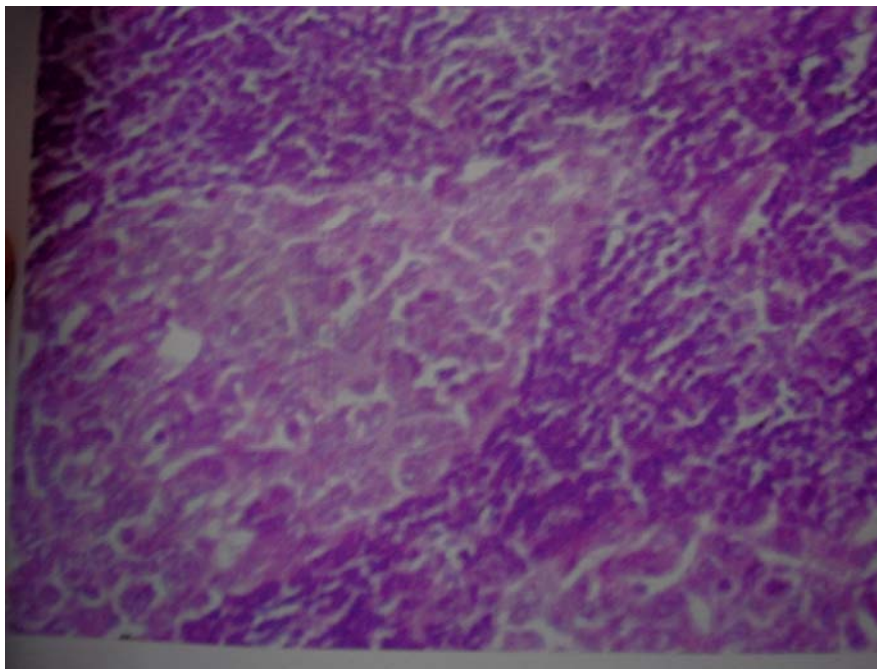
**IMPRINT SMEAR - PARA AORTIC NODE**



**IMPRINT SMEAR - INGUINAL NODE**



**HISTOPATHOLOGY - PARA AORTIC NODE**



**HISTOPATHOLOGY - INGUINAL NODE**



### MASTER CHART

Pt. No.	Name	Age/Sex	Path. No.	Primary Cancer	No. Of Imprint	Node/Specimen	IC	HPE
1.	Jayanthi	36F	853/03	Ca.thyroid	1 .2	L-Hemithyroidectomy L-Paratracheal node	Negative Negative	Negative Negative
2.	Petchiammal	45 F	1214/03	Ca. Thyroid	3. 4.	L-Hemithyroidectomy L-Paratracheal node	Negative Negative	Negative Negative
3.	Malliga	45F	1028/03	Ca.Cervix	5. 6.	Para aortic node (1) Para aortic node (2)	Positive Positive	Positive Positive
4.	Kannan	58M	1029/3	Ca.Tongue	7.	R-Supraomahyoid node	Positive	Positive
5.	Chinnaian	40M	1039/03	(R) Leg - SCC	8.	R -Inguinal node	Positive	Positive
6.	Soundararajan	43 M	1059/03	Ca.Bladder	9 10.	Para aortic node (1) Para aortic node (2)	Positive Sus.	Negative Negative
7.	Raghu	65M	1199/03	Ca. Penis	11. 12.	R-Inguinal node (1) R-Inguinal node (2)	Positive Positive	Positive Positive
8.	Balaganga	44F	1219/03	Ca.Cervix	13. 14.	Para aortic node (1) Para aortic node (2)	Negative Negative	Positive Negative
9.	Alagami	44F	1301/03	Ca.Cervix	15. 16.	Para aortic node (1) Para aortic node (2)	Positive Positive	Positive Positive

Pt. No.	Name	Age/Sex	Path. No.	Primary Cancer	No. Of Imprint	Node/Specimen	IC	HPE
10.	Hemavathy	48F	1337/03	Ca. Breast	17.	R-Axillary node	Positive	Positive
11.	Meenakshiammal	55F	1362/03	Ca.Stomach	18.	Para aortic node	Negative	Negative
12.	Chadrsekhar	44M	254/04	Ca.Penis	19.	R -Inguina node (1)	Negative	Positive
					20.	R -Inguinal node (2)	Negative	Negative
					21.	R - Inguinal node (3)	Negative	Positive
13.	Vijaya	40F	263/04	Ca. Breast	22.	R-Axillary node	Negative	Negative
14.	Noorjahan	40F	281/04	Ca.Breast	23.	R-Axillary node	Positive	Positive
15.	Chandrasekaran	46M	291/04	Ca.Penis	24.	L-Inguinal node (1)	Negative	Positive
					25.	L-Inguinal node (2)	Negative	Negative
16.	Dhanalakshmi	35 F	295/04	Ca. Cervix	26.	Para aortic node (1)	Positive	Positive
					27.	Para aortic node (2)	Positive	Positive
17.	Anthony	60 M	308/04	Ca. Penis	28.	L-Inguinal node (1)	Positive	Positive
					29.	L-Inguinal node (2)	Negative	Positive
					30.	L-Inguinal node (3)	Negative	Negative.
18.	Anthony	50 M	421/04	Ca. Penis	31.	L- Inguinal node (1)	Negative	Positive
					32.	L-Inguinal node (2)	Negative	Positive
19.	Kanniammal	66 F	526/04	Ca. Cervix	33.	Para aortic node	Negative	Negative

Pt. No.	Name	Age/Sex	Path. No.	Primary Cancer	No. Of Imprint	Node/Specimen	IC	HPE
					34.	Para aortic node	Negative	Negative
20.	Elumalai	58 M	575/04	Ca. Bladder	35.	R-Ext. Iliac node	Positive	Positive
21.	Neelamma	40F	709/04	Ca. Breast	36.	R-Axillary node	Positive	Positive
22.	Vimala	27F	683/04	Ca. Thyroid	37.	L-Hemi Thyroidectomy	Positive	Positive
23.	Badrunisha	46F	714/04	Ca. Breast	38.	R-Axillary node	Negative	Negative
24.	Sameer	32 M	728/04	Ca. Tongue	39.	R-Supraomohyoid node (1)	Positive	Positive
					40.	R-Supraomohyoid node (2)	Positive	Positive
25.	Kamatchi	55 F	707/04	Ca. Tongue	41.	R-Supraomohyoid node	Positive	Positive
26.	Papammal	38F	741/04	Ca. Cervix	42.	Para aortic node - (1)	Negative	Positive
					43.	Para aortic node - (2)	Negative	Positive
27.	Chandra	55F	778/04	Ca. Breast	44.	L-Axillary node	Negative	Positive
28.	Poongothai	28 F	834/04	Ca. Thyroid	45.	L-Hemithyroidectomy	Negative	Negative
29.	Gangeyan	65M	802/04	Ca. Cheek	46.	L-Supraomohyoid node	Negative	Positive
30.	Ram	70 M	869/04	Ca. Penis	47.	L-Inguinal node	Negative	Positive
31.	Lourdarmay	55 F	880/04	Ca. Breast	48.	R-Axillary node - (1)	Positive	Positive
					49.	R-Axillary node - (2)	Positive	Positive
					50.	R-Axillary node - (3)	Positive	Positive

<b>Pt. No.</b>	<b>Name</b>	<b>Age/Sex</b>	<b>Path. No.</b>	<b>Primary Cancer</b>	<b>No. Of Imprint</b>	<b>Node/Specimen</b>	<b>IC</b>	<b>HPE</b>
32.	Raman	60 M	883/04	Ca. Stomach	51. 52.	Para aortic node (1) Para aortic node (2)	Sus. Negative	Negative Negative
33.	Selvammal	47 F	885/04	Ca. Breast	53.	L-Axillary node	Negative	Negative
34.	Indiramma	55 F	900/04	Ca. Breast	54. 55. 56.	L-Axillary node - (1) L-Axillary node - (2) L-Axillary node - (3)	Positive Positive Positive	Positive Positive Positive
35.	Raman	70 M	901/04	Hodgkins Lymphoma	57.	L-Axillary node	Sus.	Positive
36.	Ganesan	17 M	940/04	Hodgkins Lymphoma	58	L-Cervical node	Sus.	Positive
37.	Kannan	54 M	944/04	Ca. Tongue	59. 60.	L-Supraomohyoid node (2) L-Supraomohyoid node (3)	Positive Positive	Positive Positive
38.	Dhanalakshmi	35 F	958/04	Ca. Thyroid	61. 62.	R-Hemithyroidectomy R-Paratreacheal node	Negative Negative	Negative Negative
39.	Selvaraj	45 M	1425/04	Lymphoma	63. 64.	L-Inguinal node L-Inguinal node	Negative Negative	Negative Negative
40.	Manikkam	40/M	1109/05	Testicular Tumour	65.	Lt. Pelvic Node	Negative	Negative

<b>Pt. No.</b>	<b>Name</b>	<b>Age/Sex</b>	<b>Path. No.</b>	<b>Primary Cancer</b>	<b>No. Of Imprint</b>	<b>Node/Specimen</b>	<b>IC</b>	<b>HPE</b>
41.	Ayyammal	55/F	1232/05	Melenona Rt. Foot	66.	Rt. Inguinal Node	Negative	Negative
42.	Viji	25/F	1242/05	Ca.Thyroid	67.	Hemithyroidectomy	Negative	Negative
43.	Tarabai	40/F	683/05	Ca.Cervix	68.	Para aortic node	Negative	Negative
44.	Ravanammal	45/F	1077/05	Ca.Cervix	69.	Para aortic node	Negative	Negative
45.	Akbar Ali	45/M	1160/05	Ca. Stomach	70.	Para aortic node	Positive	Positive

## **RESULTS AND OBSERVATIONS**

## RESULTS

### CASE DISTRIBUTION – DIAGNOSIS BASED

Diagnosis	No.	Percentage
Ca Breast.	9	20
Ca. Cervix	8	17
Ca. Penis	6	14
Ca. Thyroid	6	14
Oral Cancer	5	11
Lymphoma	3	7
Ca.Bladder	2	4
Skin Tumor	2	4
Ca. Stomach	3	7
Testicular Tumor	1	2
	44	100

### CASE DISTRIBUTION – SPECIMEN BASED

SPECIMEN	NO.	PERCENTAGE
Para aortic nodes	12	25
Inguinal nodes	9	20
Axillary Nodes	9	20
Cervical Nodes	7	16
Hemi Thyroidectomy	6	14
Pelvic Nodes	2	5
	45	100

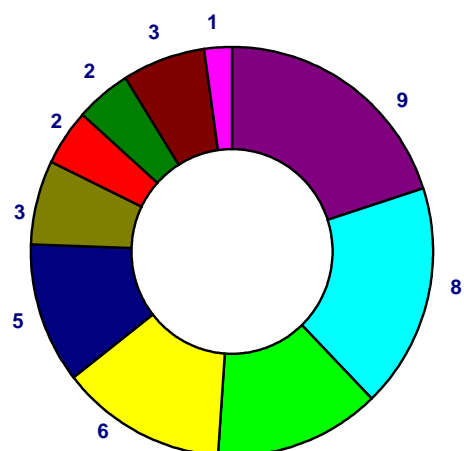
### CASE DISTRIBUTION

#### NO. OF NODE/SPECIMEN SLIDE BASED

Region	Count	Percent
Axillae	14	20
Cervical	9	13
Inguinal	17	24
Para-aortic	20	29
Pelvic	2	3
Thyroid	8	11
	<b>70</b>	<b>100</b>

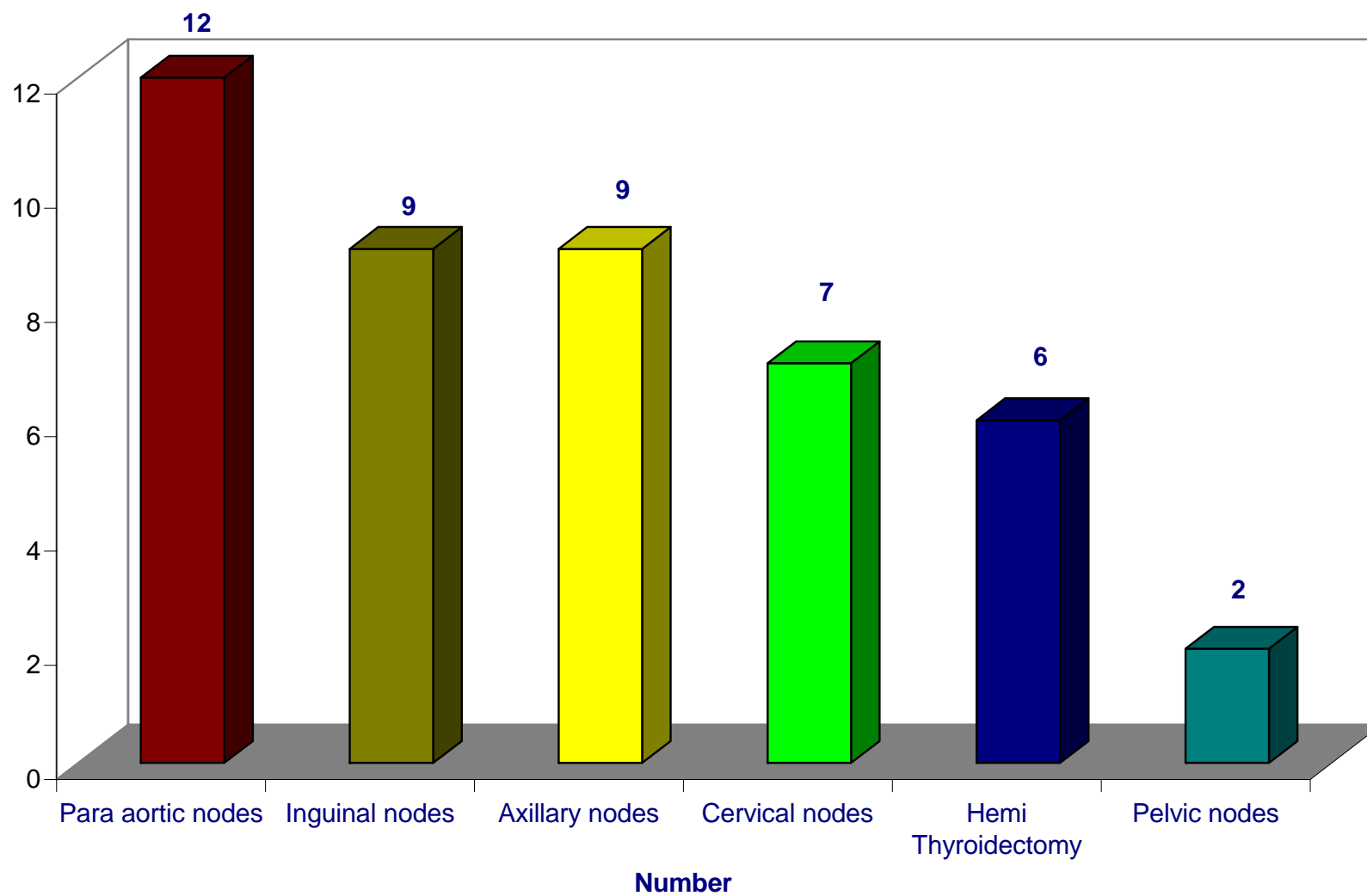


## CASE DISTRIBUTION - DIAGNOSIS BASED

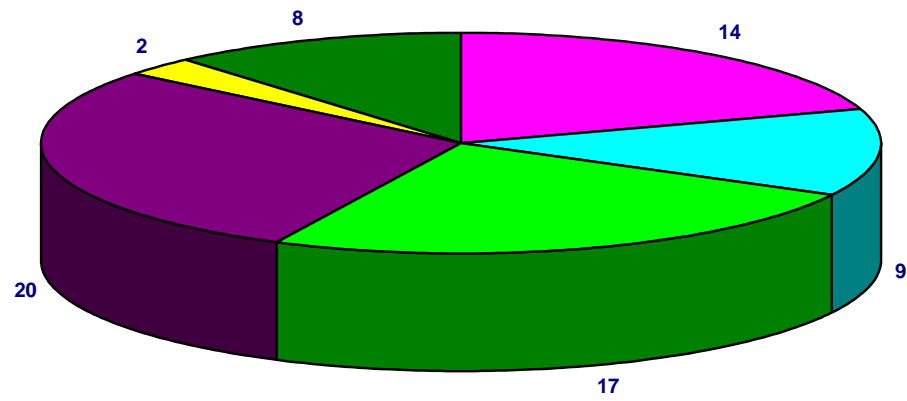


■ Ca. Breast ■ Ca. Cervix ■ Ca. Penis ■ Ca. Thyroid ■ Oral Cancer ■ Lymphoma ■ Ca. Bladder ■ Skin Tumor ■ Ca. Stomach ■ Testicular Tumor

### CASE DISTRIBUTION - SPECIMEN BASED



CASE DISTRIBUTION - NO.OF IMPRINT SLIDE BASED



## RESULTS

Out of 5 supra-omohyoid block dissections done for oral cancers, 4 were positive in imprint cytology and the dissection extended to M.R.N.D.

Out of 8 superficial inguinal block dissection (5 cases for Ca. Penis and 3 cases for skin tumor) 3 were positive and were treated by I.I.B.D.

In 6 thyroid swellings, 5 were negative for malignancy and 1 was positive and was treated by total thyroidectomy.

8 post RT Ca Cervix cases were planned for pelvic exenteration. In imprint cytology 3 patients had para aortic nodal metastasis and the procedures were done only in the remaining 5 cases.

In 3 Gastric cancers, 2 cases underwent D<sub>2</sub> gastrectomy after ruling out the para aortic nodal metastases. In 1 case it was positive and was treated by palliative gastric resection

## OBSERVATIONS FROM THE STUDY

Primary Site Lesion	Specimen	Node No.	IC	HPE	Result Category.
Thyroid	Thyroid	1	n	n	TN
Thyroid	Thyroid	2	n	n	TN
Thyroid	Thyroid	3	n	n	TN
Thyroid	Thyroid	4	n	n	TN
Cervix	Para-aortic	5	p	p	TP
Cervix	Para-aortic	6	p	p	TP
Leg	Inguinal	8	p	p	TP
Bladder	Para-aortic	9	p	n	FP
Bladder	Para-aortic	10	n	n	TN
Penis	Inguinal	11	p	p	TP
Penis	Inguinal	12	p	p	TP
Cervix	Para-aortic	13	n	p	FN
Cervix	Para-aortic	14	n	n	TN
Cervix	Para-aortic	15	p	p	TP
Cervix	Para-aortic	16	p	p	TP
Breast	Axillae	17	p	p	TP
Stomach	Para Aortic	18	n	n	TN
Penis	Inguinal	19	n	p	FN
Penis	Inguinal	20	n	p	TN
Penis	Inguinal	21	n	p	FN
Breast	Axillae	22	n	p	TN
Breast	Axillae	23	p	p	TP
Penis	Inguinal	24	n	p	FN
Penis	Inguinal	25	n	n	TN
Cervix	Para Aortic	26	p	p	TP
Cervix	Para Aortic	27	p	p	TP
Penis	Inguinal	28	p	p	TP

<b>Primary Site Lesion</b>	<b>Specimen</b>	<b>Node No.</b>	<b>IC</b>	<b>HPE</b>	<b>Result Category.</b>
Penis	Inguinal	29	n	p	FN
Penis	Inguinal	30	n	n	TN
Penis	Inguinal	32	n	p	FN
Penis	Inguinal	32	n	p	FN
Cervix	Para Aortic	33	n	n	TN
Cervix	Para Aortic	34	n	n	TN
Bladder	Pelvic	35	p	p	TP
Breast	Axillae	36	p	p	TP
Thyroid	Thyroid	37	p	p	TP
Breast	Axilla	38	n	n	TN
Tongue	Cervical	39	p	p	TP
Tongue	Cervical	40	p	p	TP
Tongue	Cervical	41	p	p	TP
Cervix	Para Aortic	42	p	p	TP
Cervix	Para Aortic	43	n	p	FN
Breast	Axillaa	44	n	p	FN
Thyroid	Thyroid	45	n	n	TN
Cheek	Cervical	46	n	n	TN
Penis	Inguinal	47	n	p	FN
Breast	Axillae	48	p	p	TP
Breast	Axillae	49	p	p	TP
Breast	Axillae	50	p	p	TP
Stomach	Para-aortic	51	n	n	TN
Stomach	Para-aortic	42	n	n	TN
Breast	Axillae	53	n	n	TN
Breast	Axillae	54	p	p	TP
Breast	Axillae	55	p	p	TP
Breast	Axillae	46	p	p	TP
Lymphoma	Axillae	57	n	p	FN

<b>Primary Site Lesion</b>	<b>Specimen</b>	<b>Node No.</b>	<b>IC</b>	<b>HPE</b>	<b>Result Category.</b>
Lymphoma	Cervical	58	n	p	FN
Tongue	Cervical	59	p	p	TP
Tongue	Cervical	60	p	p	TP
Thyroid	Thyroid	61	n	n	TN
Thyroid	Thyroid	62	n	n	TN
Lymphoma	Inguinal	63	n	n	TN
Lymphoma	Inguinal	64	n	n	TN
Testis	Pelvic	65	n	n	TN
Skin	Inguinal	66	n	n	TN
Thyroid	Thyroid	67	n	n	TN
Cervix	Para aortic	68	n	n	TN
Cervix	Para aortic	69	n	n	TN
Stomach	Para aortic	70	p	p	TP

TN - True Negative  
 FN - False Negative  
 n - Negative

TP - True Positive  
 FP - False Positive  
 p - Positive

### NAME LIST - TRUE POSITIVE NODES

Patient No.	Name	Node No.	IC	HPE
3	Mallika	5	p	p
3	Mallika	6	p	p
4	Kannan	7	p	p
5	Chinnian	8	p	p
7	Raghu	11	p	p
7	Raghu	12	p	p
9	Alagammai	15	p	p
9	Alagammai	16	p	p
10	Hemavathy	17	p	p
14	Noorjehan	23	p	p
16	Dhanalakshmi	27	p	p
17	Antony	28	p	p
20	Elumalai	35	p	p
21	Neelamma	36	p	p
22	Vimala	37	p	p
24	Sammer	39	p	p
24	Sammer	40	p	p
25	Kamakshi	41	p	p
26	Pappammal	42	p	p
31	Lourdhamary	48	p	p
31	Lourdhamary	49	p	p
31	Lourdhamary	50	p	p
34	Indramma	54	p	p
34	Indramma	55	p	p
34	Indramma	56	p	p
37	Kannan	59	p	p
37	Kannan	60	p	p
45	Akbar ali	70	p	p



### NAME LIST - TRUE NEGATIVE NODES

Patient No.	Name	Node No	IC	HPE
1	Jeyanthi	1	n	n
1	Jeyanthi	2	n	n
2	Petchiammal	3	n	n
2	Petchiammal	4	n	n
6	Sounderarajan	10	n	n
8	Balaganga	14	n	n
11	Meenakshiammal	18	n	n
12	Chandrashekar	20	n	n
13	Vijaya	22	n	n
15	Chandrashekar	25	n	n
17	Antony	30	n	n
19	Kanniammal	33	n	n
19	Kanniammal	34	n	n
23	Badhrunnisa	38	n	n
25	Poongothai	45	n	n
29	Kangeyan	46	n	n
32	Raman	51	n	n
32	Raman	52	n	n
33	Selvammal	53	n	n
38	Dhanalakshmi	61	n	n
38	Dhanalakshmi	62	n	n
39	Selvaraj	63	n	n
39	Selvaraj	64	n	n
40	Manikkan	65	n	n
41	Ayyammal	66	n	n
42	Viji	67	n	n
43	Tarabai	68	n	n
44	Ravanammal	69	n	n

### NAME LIST - FALSE NEGATIVE NODES

Patient No.	Name	Node No.	IC	HPE
8	Balaganga	13	n	p
12	Chandrashekar	19	n	p
12	Chandrashekar	21	n	p
15	Chadnrashakar	24	n	p
17	Antony	29	n	p
18	Antony	31	n	p
18	Antony	32	n	p
26	Pappammal	42	n	p
26	Pappammal	43	n	p
27	Chandra	44	n	p
30	Ram	47	n	p
35	Raman	57	n	p
36	Ganesan	58	n	p

### NAME LIST - FALSE POSITIVE NODES

Patient No.	Name	Node No.	IC	HPE
6	Sounderarajan	9	p	n

n - Negative

p - Positive

## **DISCUSSION**

## DISCUSSION

In this prospective study, we compared the diagnostic accuracy of Touch Imprint Cytology in 45 patients on 70 slides, which were suspected to harbor pathology for the assessment of histological status. All the patients had a definitive histological diagnosis before inclusion into this study.

### NO. OF NODE/SPECIMEN SLIDE BASED

Region	Count	Percent
Axillae	14	20
Cervical	9	13
Inguinal	17	24
Para-aortic	20	29
Pelvic	2	3
Thyroid	8	11
	70	100

In total, of the sample assessed from different regions of 45 patients by comparing Intra Operative Touch Imprint cytodiagnosis work up followed by definitive paraffin sections, we were able to categorize these nodes as follows.

- I. No of True positive: 29
- II. No of True negative: 27
- III. No of False negative: 13
- IV. No of False positive: 01

The sensitivity, specificity, accuracy, positive and negative predictive value of Imprint Cytology were calculated relative to final pathologic status as follows.

$$\text{Sensitivity} = \frac{\text{TP}}{\text{TP} + \text{FN}}$$

$$\frac{29}{29+13} = \frac{29}{42} = 69.05\%$$

$$\text{Specificity} = \frac{\text{TN}}{\text{TN} + \text{FP}}$$

$$\frac{27}{27+1} = \frac{27}{28} = 96.43\%$$

$$\text{PPV} = \frac{\text{TP}}{\text{TP} + \text{FP}}$$

$$\frac{29}{29+1} = \frac{29}{30} = 96.67\%$$

$$\text{NPV} = \frac{\text{TN}}{\text{TN} + \text{FN}}$$

$$\frac{27}{27+13} = \frac{27}{40} = 67.50\%$$

$$\text{Accuracy} = \frac{\text{TP} + \text{TN}}{\text{TP} + \text{FP} + \text{FN} + \text{TN}}$$

$$\frac{27}{29+1+13+27} = \frac{56}{70} = 80\%$$

(NPV – Negative predictive value)

(PPV – Positive predictive value)

**Results were :**

A.	Sensitivity	-	69.05%
B.	Specificity	-	96.43%
C.	Positive predictive value (PPV)	-	96.67%
D.	Negative predictive value (NPV)	-	67.50%
E.	Accuracy	-	80.00%

The sensitivity and specificity are important factors in deciding whether a technique should be used. The sensitivity of Touch Imprint cytology in our study is 69.05% which is moderately sensitive in comparison to the published series ranging from 38 to 96%.

The wide disparity between the results of these published studies cannot be explained by the methodology alone, but rather due to observer variations. Since our protocol requirement is prefixed as one imprint from each half of bisected lymph node and also the lymph nodes taken up for the study were from a heterogeneous population of sites with a different possible histology of the primary cancer, this could have contributed to a moderately lower degree of sensitivity.

When the data were examined on a per lymph node basis, of the 70 nodes studied, 42 (60%) nodes harbored metastatic carcinoma on permanent section evaluation. If paraffin sections were negative, serial sections were done to rule out the presence of occult metastases in the deeper sections. Of these, 29 were detected on Imprint cytology, resulting in a sensitivity of 69.05%. The negative predictive value was 67.50%

To minimize the possibility of false positive results at our institution, intraoperative indeterminate (atypical / suspicious) results, are regarded as negative at the time of surgery and at final diagnosis, unless confirmed positive on paraffin section. In our four indeterminate results, 2 turned out to be positive and two were negative on final paraffin section. The two cases which were negative on the final paraffin section illustrate that reactive histiocytes, lymphocytes, and endothelial cells may appear atypical and may be difficult to

distinguish from a micro metastasis. Retrospective review of some published series confirms that indeterminate results seemed more common with imprint cytology than with frozen section, suggesting that this may be a greater problem with imprint cytology.

### **SENSITIVITY OF IMPRINT CYTOLOGY IN LITERATURE**

<b>First Author</b>	<b>Year</b>	<b>No. Of Patients</b>	<b>Sensitivity %</b>
Ku	1999	76	22
Rubio	1998	53	96
Van Diest	1999	54	64
Motomura	2000	101	91
Ratanawishitrasi	1999	55	93
Llatjos	2002	76	68
Henry – Tillman	2002	247	94
Cserni	2001	60	59
Baitchev	2002	87	83
Creager	2002	646	98
Shiver	2002	127	100
Lee	2002	65	98
Barranger	2003	180	98
Present Study	2005	44	69.05

In our study, a node from the Para aortic region in a patient with carcinoma of bladder contributed to a false positive result. The interpretation of isolated tumor cell in that node was due to the presence of an atypical reactive lymphocyte, which is also discussed quite extensively in few studies. Hence



this one false positive case contributed to a mild decrease in our specificity and positive predictive value compared to that published series.

The accuracy in present study is 80%, which is in the reference range of the most widely acclaimed studies published earlier (75 – 99%).

### **Possible pitfalls in this study**

1. The sample size is small to statistically signify the higher false negative rate . Further the sample of nodes taken up for the study is not equally distributed among the various regions analyzed.
2. Since the nodes taken up for the study is heterogeneous in distribution, extrapolating the rich axillary nodal experience to other sites should be made with caution.
3. Direct comparison of frozen section could have further bolstered our finding in this study.
4. The use of immunohistochemical staining with CK – IHC could have enhanced the sensitivity and deflated the false negative rate in this study.

## CONCLUSION

## CONCLUSION

The results of the current study demonstrate that the accuracy of Imprint cytology is high enough to warrant its use for Intraoperative pathological assessment. It can be accomplished during primary surgery and does not appear to prolong the surgical procedure significantly. The efficacy of the method is good. The main limitation of such an approach is an obviously low sensitivity for the detection of micro metastases.

From the above discussion, it is clear that many gaps remain in our knowledge of optimal pathologic analysis of nodal metastases, but some tentative general conclusions can be drawn – which may be invalidated by subsequent, more definitive studies.

Intraoperative pathologic assessment of nodal status should be a joint decision between the surgeon and the pathologist, based on the likelihood of metastases and the relative risks and benefits to the patient in a given case.

Intraoperative cytological Touch imprint on bisected nodes is a reasonable approach to identify at least a proportion of the larger metastases, without wasting significant lymph node tissue. It should be understood that micro metastases are unlikely to be detected by this method, however.

Imprint cytology is a viable alternative to traditional frozen section when intraoperative evaluation is required. Given its 96.67% specificity surgeon may feel confident in basing their Intraoperative decision on touch imprint cytology results. Clearly, Imprint cytology evaluation of lymph nodal

status lacks sufficient sensitivity to serve as the final diagnostic test. Further evaluation of permanent sections using traditional techniques such as H & E still remains the gold standard and is indicated.

Despite the simplicity, speed and excellent cellular detail the technique has still not been fully utilized. We advocate the consideration of Touch imprint cytodiagnosis for the evaluation of cases with nodal metastases as a valuable alternative to frozen section histology.

# **PROFORMA**

## PROFORMA - IMPRINT CYTOLOGY

**Name** : **Age / Sex**

**Occupation** :

**Address** :

**M.R.D NO.** : **C. D. No.**

**Diagnosis** :

**Site of Primary:**

**Stage-cTNM** :

**Imprint cytology** :  
**Site of node**  
**No of nodes**  
**Report**

**Surgery according  
to imprint cytology** :

**Corresponding HPE** :

**Stage - pTNM** :

**Further treatment :**

**Follow up :**

### **CASE DETAILS**

**Complaints :**

**Present History :**

**Past History :**

**General Examination :**

**Local examination :**

**Systemic examination :**

**Investigations :**

**X-ray :**

**Ultrasound :**

**CT scan :**

**Other Investigations :**

# **BIBLIOGRAPHY**



## BIBLIOGRAPHY

1. Shiver SA, Creager AJ, Geisinger K, Perrier ND, Perry shen, Levine EA. Intraoperative analysis of sentinel Lymph nodes by Imprint cytology for cancer of the breast. *Am J Surgery* **184** (2002) 424-427.
2. Creager AJ, Shiver SA, Perry Shen, Geisinger KR, Edward A.Levine. Intra operative Evaluation of Sentinel Lymph nodes for Metastatic Melanoma by Imprint Cytology. *Cancer June 1, 2002 / Vol.94/ Number 11*.
3. Turner, Hansen NM, Stern SL, Giuliano E. Intraoperative Examination of the Sentinel Lymph node for Breast Carcinoma staging. *Am J Clin Pathol* **1999; 112; 627 - 634**.
4. Baitcher, Gortcher, Todorova. Intra opeative sentinel lymph node examination by Imprint cytology during breast surgery. *Current Medical Research and Opinion* **Vol.18, No.4, 2002, 185-187**.
5. Mullnex, Carter, Martin, Steele, Scott, Watts, Beitler. Predictive value of intra operative touch preparation analysis of sentinel lymph nodes for axillary metastasis in breast cancer. *Am J Surgery* **185** (2003) 420 - 424.
6. Ratanawichitrasin A, Biscotti CV, Levy L, Crowe JP. Touch Imprint cytological analysis of Sentinel lymph nodes for detecting axillary metastasis in patients with breast cancer. *B J Serg.* **1999 Oct; 86 (10); 1346 - 8**.
7. Tara Karamlou, M.D., Nathalie M. Johnson, M.D. Benjamin Chan, M.S., Daisy Franzini, M.D. Diana Mahin, C.T.R. Accuracy of intraoperative touch imprint cytologic analysis of sentinel lymph nodes in breast cancer. *The American Journal of Surgery* **185** (2003) 425 - 428.
8. Van Diest PJ, Torrensa H, Borgstein PJ, Pijpers R, Bleichrodt R Rahusen FD, Meijer S. Reliability of intraoperative frozen section and imprint cytological investigation of sentinel lymph nodes in breast cancer. *Histopathology.* **1999 July; 35(1); 14-8**.
9. Melissa A. Bochner, M.S., Gelareh Farshid, M.I.A.C., Thomas J. Dodd, (Hons.), James Kollias, P.Grantley Gill, M.D. Intraoperative Imprint cytologic Assessment of the Sentinel node for Early Breast Cancer. *World J. Surg.* **27, 430 - 432, 2003**.

10. P.A. Lambath, M.A. McIntyre, U. Chetty and J.M. Dixont. Imprint cytology of axillary lymph nodes as an intraoperative diagnostic tool. ***EJSO* 2003; 29: 224 - 228.**
11. Salem AA, Douglas - Jones AG, Sweetland HM, Newcombe RG, Mansel RE. Evaluation of axillary lymph nodes using touch imprint cytology and immunohistochemistry. ***Br.J Surg.* 2002 Nov; 89(11): 1386-9.**
12. M.L. Smidt, R.Besseling, C.A.P. Wauters and L.J.A. Strobbe. Intraoperative scrape cytology of the sentinel lymph node in patients with breast cancer. ***British Journal of Surgery*, 2002, 89, 1290 - 1293.**
13. Zgajnar, J, Frkovi - Grazio S, Besic N, Hocevar M, Vidergarkralk B, Gerljvic A, Pogacink A. Low sensitivity of the touch imprint cytology of the sentinel lymph node in breast cancer patients - results of a large series. ***J. Surg Oncol.* 2004 Feb; 85(2): 82 - 61.**
14. Emmanuel Barranger, Martine Antoine, Dany Grahek, Partice Callard, Serge Uzan. Intraoperative Imprint Cytology of Sentinel Nodes in Breast Cancer. ***Journal of Surgical Oncology* 2004; 86: 128 - 133.**
15. Creager AJ, Shaw AJ, Young PR, Geisinger KR. Intraoperative evaluation of lumpectomy margins by imprint cytology with histological correlation; a community hospital experience. ***Arch Pathol Lab Med.* 2002 Jul; 126(7) : 846 - 8.**
16. Prem Sharam, Vatasla Misra, PA Singh, SP Misra, SC Gupta. A Correlative Study of Histology and Imprint Cytology in the Diagnosis of Gastrointestinal Tract Malignancies. ***Indian J. Pathol. Microbiol.* 0(2): 139 - 146, 1997.**
17. Ahmed S. Shabaik, Charles E. Cox, Robert, A. Clark, Douglas S. Reintgen, Edwin J. Humphrey, Santo V. Nicosia. Imprint Cytology of Needle - Localized Breast Lesions. ***Acta Cytologica* Vol.37 ;( 1) Jan - Feb 1993.**
18. Bhabra K, Goulden RG, Peel KR. Intra-operative diagnosis of lymph node metastases in gynaecological practice using imprint cytology. ***Eur J Gynaecol Oncol.* 1989; 10(2): 117 - 24.**
19. Patrick A. Treseler, and Pamela S. Tauchim. Pathologic Analysis of the Sentinel Lymph Node. ***Surgical Clinics of North America.* Vol. 80(6) December 2000.**

20. A.A. Salem, A.G. Douglas - Jones, H.M. Sweetland and R.E. Mansel. Intraoperative evaluation of axillary sentinel lymph nodes using touch imprint cytology and immunohistochemistry. I. Protocol of rapid immunostaining of touch imprints. *EJSO* 2003; 29: 25 - 28.
21. Kumar Dutta, Amitabha Chattopadhyaya, Shrivasthi Roy. Evaluation of fine Needle Aspiration and Imprint cytology in the early Diagnosis of breast Lesions with Histopathological correlation. *J. Indian Med Assoc*, Vol. 99, No.88, August, 2001.
22. Andrea Lee, Savitri Krishnamurthy, Aysegul Sahin, W., Fraser Symmans, Kelly Hunt, Nour Senige. Intraoperative Touch imprint of Sentinel Lymph Nodes in Breast Carcinoma Patients. *Cancer* August 25, 2005 / Vol. 96 (4).
23. Motomura K, Inaji H, Komoike Y, Kasugai T, Nagumo S, Noguchi S, Koyama H. Intraoperative sentinel lymph node examination by imprint cytology and frozen sectioning during breast surgery. *Br.J. Surg.* 2000 May; 87 (5): 597 - 601.
24. Llatjos, Castella, Fraile, Rull, Julian, Fuste, Rovira, Fernandez-Llamazares. Intraoperative Assessment of Sentinel Lymph nodes in Patients with Breast Carcinoma. *Cancer* June 25, 2002 Vol. 96(3).
25. Viale, Bosari, Mazzarol, Galimberti, Luini, Veronesi, Paganelli, Bedoni, Orvieto, Intraoperative Examination of Axillary Sentinel Lymph Nodes in Breast Carcinoma Patients. *Cancer* June 1, 1999 Vol. 85 (11).
26. Champakam, Vasco D' Souza, J.R. Kamat. Imprint cytology in the Diagnosis of Breast Tumor. *INT Surg* 1982; 67: 425 - 426.
27. Roderick R.Turner, David W. Ollila, Stacey Stern, and Armando E. Giuliano. Optimal Histopathologic Examination of the Sentinel Lymph Node for Breast Carcinoma Staging. *The American Journal of Surgical Pathology* 23(3): 263 - 267. 1999.
28. Ni Ni K. Ku, Nazeel Ahmad, Prudence V. Smith, Charles E. Cox Alan Shons, Douglas S. Reintgen, Santo V. Nicosia. Intraoperative Imprint Cytology of Sentinel Lymph Nodes in Breast Cancer. *Acta Cytologica* 41, (5) Sept - Octo 1997.
29. Asthana S, Deo SV, Shukla NK, Jain P, Anand M, Kumar R. Intraoperative neck staging using sentinel node biopsy and imprint cytology in oral cancer. *Head Neck.* 2003 May; 25(5): 368 - 72.

30. MR Clarke, RJ Landreneau and D Borochoviz. Intraoperative imprint cytology for evaluation of mediastinal lymphadenopathy. *The Annals of Thoracic Surgery*, Vol. 57, 1206 - 1210.
31. Quill DS, Leahy AL, Lawler RG, Finney RD. Lymph node imprint cytology for the rapid assessment of axillary node metastases in breast cancer. *Br. J Surg.* 1984 Jun; 71(6): 454 - 5.
32. Suen KC, Wood WS, Syed AA, Quenmville NF, Clement PB. Role of imprint cytology in intraoperative diagnosis: value and limitations. *J Clin Pathol.* 1978 Apr; 31(4) 328 - 37.
33. Kim K, Phillips ER, Paolino M. Intraoperative imprint cytology: its significance as a diagnostic adjunct. *Diagn Cytopathol.* 1990; 6 (5) : 304 - 7.
34. Molyneux AJ, Attanoos RL, Coghill SB. The value of lymph node imprint cytodiagnosis : an assessment of interobserver agreement and diagnostic accuracy. *Cytopathology.* 1997 Aug; 8 (4) 256 - 64.
35. Scopa CD, Melachrinou M, A pessou D, Bonikos D. Tissue imprints in surgical pathology; a rapid intraoperative diagnostic aid. *Diagn Cytopathol* 1990 6 (1) 5 - 8.
36. Aryya NC, Khanna S, Shukla HS, Tripathi FM, Shukla VK. Role of rapid imprint cytology in the diagnosis of skin cancer and assessment of adequacy of excision. *Indian J Pathol Microbiol.* 1992 Apr; 35 (2): 108 - 12.
37. John D. Bancroft., Marilyn Gamble. Theory and Practice of histological techniques. Fifth Edition.
38. **John Ogle, MD, MPH, FACEP**, Department of Public Health, Yale University School of Medicine; Medicine - Introductory Biostatistics.